Journal of Chromatography, 237 (1982) 99–105
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,442

DETERMINATION OF CHROMIUM IN ORCHARD LEAVES BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

JAMES D. WILLETT and MARGARET M. KNIGHT*,*
Department of Chemistry, University of Idaho, Moscow, ID 83843 (U.S.A.)
(Received October 6th, 1981)

SUMMARY

Chromium, as chromium acetylacetonate, was determined in nitric acid digested samples of NBS Standard Reference Material 1571, Orchard Leaf by reversed-phase high-performance liquid chromatography on μBondapak C_{18} using 36% acetonitrile in water. The determined value was 2.4 ± 0.1 ppm. The limits of detection for this method are 1 ng on-column injection. By standard microchemical enrichment techniques parts-per-billion analyses can be achieved.

INTRODUCTION

Gas chromatography and, more recently, high-performance liquid chromatography (HPLC) have been used with increasing frequency for the separation of metal complexes since their separation by gas chromatography was first reported in 1959[1–3]. The extraction of metals by chelating agents in organic solvents is a well established procedure. By carefully selecting ligand, solvent, pH of the aqueous phase, and sequestering agents, one can enhance or modulate the preferential extraction of a specific metal or group of metals from a complex matrix. Efficient chromatographic separation coupled with selective solvent extraction of metal complexes from matrices of interest provides a system capable of rapid, simultaneous multi-element analysis of the trace or ultratrace level. Advantages of the use of chromatography of metal complexes over other methods capable of trace metal analysis such as atomic absorption, emission spectrometry, and neutron activation analysis include the possibility of distinguishing among oxidation states of a given metal and distinguishing free versus bound metals in various matrices. The decreased expense of chromatographic analysis relative to at least some of the alternatives may prove advantageous in some instances.

Gas chromatography has been used for the quantitative analysis of a very few metals as metal chelates from biological and inorganic matrices[4–14]. However, the requirements of the gas chromatographic analysis of metal complexes, volatility and thermal stability, are not inherent characteristics of metal complexes themselves.

* Present address: WTC 2D25, Weyerhaeuser Company, Tacoma, WA 98477, U.S.A.

0021-9673/82/0000–0000/$02.75

Even fluorinated β-diketonates, many of which are volatile and thermally stable, undergo on-column hetero-molecular reactions in the complex molecules. These on-column interactions become especially apparent at trace levels[15–17]. HPLC on the other hand, is not dependent upon high volatility or thermal stability for efficient and effective separation. Further, increased selectivity over gas–liquid chromatography can be gained in HPLC by the added partition variable of the solvent mobile phase[16].

The analytical potential of HPLC for metals has been demonstrated. Complexes of Cr(III), Fe(II) and Mn(II)[18] have been investigated as well as tricarbonyl homologues of Cr(III)[19], triphenyl phosphine complexes of rhodium and iridium[20], acetylacetonates and trifluoroacetylacetonates of several transition metals[21,22] and tetradentate β-ketoamine complexes of Co(II), Ni(II), Cu(II), and Pd(II)[23,24]. Heizmann and co-workers[25,26] have separated several metal 1,2-diketobisthiobenzyhydrazones, dialkyldithiocarbamates, 1,2-diketobisthiosemicarbazones, and dithizonates by HPLC. Some of these complexes undergo on-column exchange reactions which create multiple peaks per metal analyzed[16,17]. However, there is a paucity of analytical determinations on real samples using the procedure.

Determinations have been made on standard solutions of diacetylbisthiobenzhydrazonates of Cu(II), Fe(II), Mn(II), Zn(II), Cd(II), and Pb(II) to 10 to 1100 ng[28]. To date, however, application of high-performance liquid chromatography to metal ion analysis in real systems has been limited to the determination of selenium as the naphthalene–2,3-diaminato complex in industrial waste, sewage sludge, and biological materials[29]. We would like to report a method for the determination of chromium in NBS Standard Reference Material 1571, Orchard Leaf, using reversed-phase HPLC. This method allows detection of chromium in biological materials at the parts-per-billion (10^9) level with an on-column detection limit of 1 ng.

EXPERIMENTAL

Liquid chromatograph

A Waters Associates ALC-202 liquid chromatograph was used with a model 6000 pump at a constant flow-rate of 1 ml/min. The chromatograph was equipped with a 254 nm detector.

Columns

Waters Associates μBondapak C_{18} and μPorasil were used.

Injection port

A Precision Sampling Valveseal septumless injector was used.

In-line filter

A removable 2-μm filter was installed between the injector and the column to minimize column plugging.

Syringes

Samples were injected using 10- or 25-μl syringes (Precision Sampling LC212B).

Mobile phase

Eluent was 36% acetonitrile in water.

Reagents

Unless otherwise specified, reagents are Mallinckrodt reagent-grade chemicals. Nitric acid was purified by sub-boiling distillation. All water used was purified with a Millipore Super Q water purification system. The metal chelates were synthesized according to standards methods[30], recrystallized, and characterized by their melting points and mass spectra. Acetylacetone (Aldrich reagent) was redistilled before use.

Glass ware

All glassware was cleaned by soaking overnight in concentrated sulphuric acid saturated with sodium nitrate and then rinsed eight times in purified water.

Analytical procedure

A 250-mg amount of Orchard Leaf was placed in a 10–50-ml round-bottomed flask equipped with a reflux condenser and 2 ml of nitric acid added. The digestion mixture was allowed to predigest overnight at room temperature, after which the temperature was gradually increased to 150°C. The sample was digested for 2 h. The solution was transferred, with water washings, to a 10-ml volumetric flask and the pH adjusted to 6.0–6.1 with solid Na_2CO_3. Both the solid and a saturated solution of Na_2CO_3 were used with success; however, it was found that when the base was stored in glass, metals leached from the glass interfered in the analysis. A 5-ml aliquot of the digest was transferred to a 3-dram vial, 100 μl of acetylacetone was added, and the vial closed with a Miniert top (Pierce Chemical). The vials were placed in a metal canister in a 100°C oven for 1 h, allowed to cool to room temperature, and 1 ml of chloroform added to the cooled solution. The immiscible phases were mixed on a Vortex mixer for 30 min and the excess acetylacetone removed from the chloroform extract by two successive washings with 1 ml 2 *M* NaOH. The chloroform layer was separated and backwashed with water. An aliquot was then transferred to a 1-ml Reactivial. Phase separation was facilitated throughout by centrifugation. The chloroform was evaporated from the aliquot under nitrogen and ethanol used to reconstitute the sample. A portion of the ethanol solution was then injected onto the HPLC column.

RESULTS AND DISCUSSION

Previous research on HPLC separation of metal acetylacetonates has emphasized the use of polar silica gel columns[21,22].

Huber *et al.*[21] used a polar liquid phase and a non-polar mobile phase to separate acetylacetonates of beryllium, copper, aluminium, chromium, ruthenium, cobalt. The ternary system consisted of isooctane, ethanol, and water. The water-rich phase was used as a stationary phase, an immiscible water-poor phase as the eluent. Hydrolysis and other chelate reactions, however, caused severe peak distortion. These reactions were suppressed by adding a trace of acetylacetone equal to 0.008 of the mass fraction of the mobile phase, 0.002 of the stationary phase. Tollinche and Risby[22] investigated a variety of commercially available silica, alumina, bonded

phase, and open pore polyurethane columns, and found silica to be the most useful for metal chelate analysis.

Our initial screening tests employed a polar column, μPorasil, and a non-polar variety of eluents. It was found that peaks were irreproducible at the nanogram level. We believe that the chelate is being strongly bound to active silanol groups on the μPorasil column. This seems to be supported by Kutal and Sievers[31] study with chromium trifluoroacetylacetonate in which isomerism was accelerated by glass surfaces and by Dilli and Patsalides[14] study on retention of various trifluoroacetylacetonates in gas chromatography columns. Adding a trace of ligand to the eluent stream, a technique used in both gas[32] and liquid chromatography[21] of metal complexes to suppress complex disassociation proved not to be useful for our purposes, since a mass fraction of even 0.002 in the mobile phase saturated the UV detector. As we found that UV-absorbing polar substances were formed during the digestion and chelation procedure which could bond strongly to polar columns and decrease column efficiency, we abandoned further separations using polar columns.

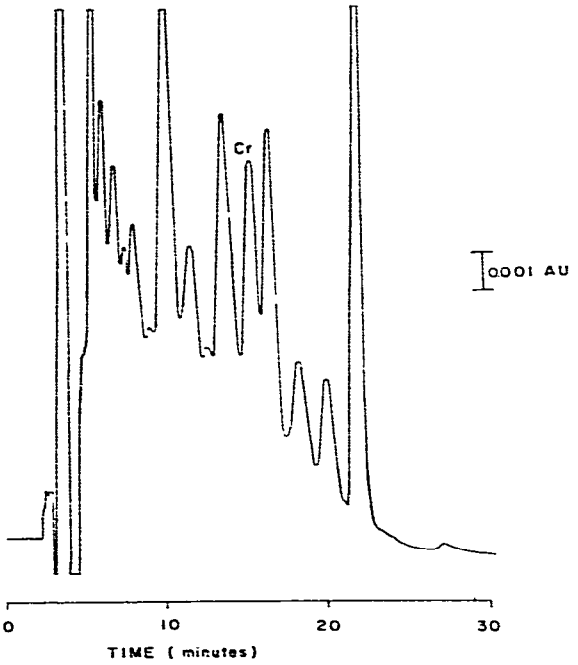
Heat is necessary for the quantitative chelation of chromium with acetylacetone[33] so reaction vessels were placed in a metal canister and a safety shield was placed in front of the oven in the event of glass failure. No difficulty was encountered with either regular glass vials or with Reactivials as long as the oven temperature remained about 100°C.

Metal analyses were run on different days with different glassware and solution to maximize variability. The glassware used was not silanized. In our initial studies we found that if the analyses were performed on freshly chelated solutions of the digests loss of chromium to the glass surface was unimportant. The acid digests showed no loss of chromium over 48 h. However, once chelated some loss of chromium to glass container walls can occur. A 13% loss of chromium was noted from a standard solution stored for 24 h. No discernable loss occurs if the solutions are used within 3–4 h of preparation.

Though chloroform was a convenient extraction solvent for the complex[33] it forms a 2:1 adduct[34] which loses chloroform on-column, complicating the analysis. Therefore we evaporated the chloroform and substituted ethanol. No loss of chromium to the glass walls was observed.

In order to ensure that the peak observed was chromium acetylacetonate, the compound was collected from the liquid chromatograph, evaporated, and analyzed by direct probe mass spectrometry. The spectrum was identical to that of the original tris(acetylacetonato) chromium(III).

Typical chromatograms for digested orchard leaf and a blank are shown in Figs. 1 and 2. Chromium is reasonably well resolved from the other peaks, presumably other metal acetylacetonates. Data for the analysis of orchard leaf is shown in Table I. The value of 2.4 ± 0.1 μg/g is within the standard deviation reported for chromium in that NBS standard of 2.6 ± 0.3 μg/g. The reproducibility of injection is shown in Table II. Response of the detector was linear over the range 1 ng (limits of detection) to 5 μg. Recovery of chromium from standard solutions is summarized in Table III. We believe that these results demonstrate the applicability of this method to the trace determination of chromium in biological materials and we have used this procedure to determine chromium in the free living nematode *Panagrellus redivivus* to less than 1 μg/g on a 20 μg sample.



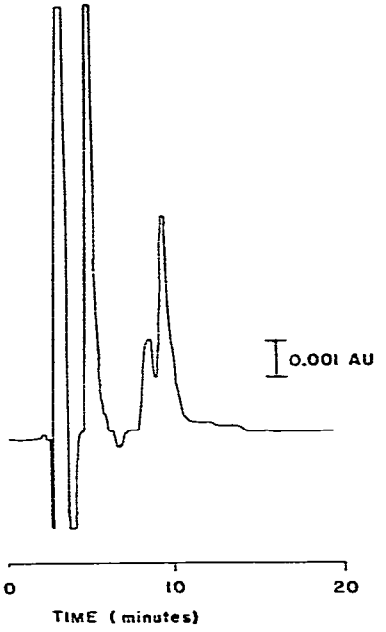


Fig. 1. Elution profile of digested orchard leaves chelated with acetylacetone. Column conditions: μBondapak C_{18} eluted with 36% acetonitrile in water at 1 ml/min.

Fig. 2. Elution profile of blank. Column conditions as in Fig. 1.

As an analysis for chromium, this method is not uniquely sensitive. Graphite furnace atomic absorption, for example, is capable of detection to 0.2 pg[35]. However, we think this system will afford rapid, single or multi-element analysis on relatively small amounts of material, depending on the exact conditions used. Acetylacetone forms well-defined chelates with over 60 metals, most of which are soluble in organic solvents and are formed under milder conditions than the chromium complex studied here[36]. We expect that conditions for extraction can be selected to optimize analysis for one metal, as in the present case, or a group of metals with minor changes in the

TABLE I

CHROMIUM IN ORCHARD LEAVES

Sample	*Weight (mg)*	*ppm*
1	250	2.3
2	250	2.5
3	250	2.3
4	250	2.5
Average		2.4 ± 0.12
Relative deviation		5%

TABLE II

REPRODUCIBILITY OF INJECTION

15 µl (15 ng)	*Peak height (mm)*
1	127
2	126
3	117
4	118
5	121
6	122
Average	122 ± 4
Relative deviation	3%

TABLE III

RECOVERY OF ADDED Cr

Added (ng)	*Found (ng)*	*Recovery (%)*
150	152.4	102
150	146.4	98
150	145.5	97
150	154.5	103
		Average 100 ± 3
		Relative deviation 3%

procedure outlined. Using standard microtechniques for enrichment by solvent extraction, trace analysis to the parts-per-billion level is expected.

REFERENCES

1 G. Guiochon and C. Pommier, *Gas Chromatography in Inorganics and Organometallics*, Ann Arbor Science, Ann Arbor, MI, 1973.
2 R. W. Moshier and R. E. Sievers, *The Gas Chromatography of Metal Chelates*, Pergamon Press, London, 1965.
3 W. D. Ross and R. E. Sievers, *Anal. Chem.*, 41 (1969) 1109.
4 W. D. Ross, R. E. Sievers and G. Wheeler, Jr., *Anal. Chem.*, 37 (1965) 598.
5 R. E. Sievers, J. W. Connolly and W. D. Ross, *J. Gas Chromatogr.*, 5 (1967) 241.
6 G. P. Morie and T. R. Sweet, *Anal. Chem.*, 37 (1965) 1552.
7 J. Savory, M. T. Glenn and J. A. Ahlstrom, *J. Chromatogr. Sci.*, 10 (1972) 247.
8 G. M. Frame, R. E. Ford, W. G. Scribner and T. Ctvrtnicek, *Anal. Chem.*, 46 (1974) 534.
9 C. A. Burgett and J. S. Fritz, *Anal. Chem.*, 44 (1972) 1738.
10 W. R. Wolf, M. L. Taylor, B. M. Hughes, T. O. Tiernan and R. E. Sievers, *Anal. Chem.*, 44 (1972) 616.
11 R. Ross and T. Shafik, *J. Chromatogr. Sci.*, 11 (1973) 46.
12 L. C. Hansen, W. G. Scribner, T. W. Gilbert and R. W. Sievers, *Anal. Chem.*, 43 (1971) 349.
13 G. H. Booth, Jr. and W. J. Darby, *Anal. Chem.*, 43 (1971) 831.
14 S. Dilli and E. Patsalides, *J. Chromatogr.*, 176 (1979) 305–318.
15 L. R. Snyder and J. J. Kirkland, *Modern Liquid Chromatography*, Wiley-Interscience, New York, 1974.
16 O. Liška, G. Guiochon and H. Colin, *J. Chromatogr.*, 171 (1979) 145–151.
17 J. Lehotay, O. Liška, E. Brandšteterová and G. Guiochon, *J. Chromatogr.*, 172 (1979) 379–383.
18 R. Eberhardt, H. Lehner and K. Schloegl, *Mg. Chem.*, 104 (1973) 1409; *Anal. Abst.*, 27 (1974) 1998.

19 H. Veening, J. M. Greenwood, W. H. Shanks and B. R. Williford, *Chem. Commun.*, (1969) 1305.
20 C. T. Enos, G. L. Geoffrey and T. H. Risby, *Anal. Chem.*, 48 (1976) 990.
21 J. F. K. Huber, J. C. Kraak and H. Veening, *Anal. Chem.*, 44 (1972) 1554.
22 C. A. Tollinche and T. H. Risby, *J. Chromatogr. Sci.*, 161 (1978) 445–454.
23 G. E. Gaelani, C. F. Laureri, A. Mangia and G. Parolari, *Anal. Chem.*, 48 (1976) 1725.
24 P. C. Uden and F. H. Walters, *Anal. Chim. Acta*, 79 (1975) 175.
25 P. Heizmann and K. Ballschmiter, *J. Chromatogr.*, 137 (1977) 153–163.
26 M. Lohmüller, P. Heizmann and K. Ballschmiter, *J. Chromatogr.*, 137 (1977) 165–170.
27 P. Heizmann and K. Ballschmiter, *Z. Anal. Chem.*, 266 (1973) 206.
28 J. W. O'Laughlin and T. P. O'Brien, *Anal. Lett.*, A11 (1978) 823–844.
29 G. L. Wheeler and P. F. Lott, *Microchem. J.*, 19 (1974) 390; *Anal. Abstr.*, 29 (1975) 22.
30 W. L. Fernelius and J. E. Blanch, *Inorg. Snyth.*, 5 (1957) 131.
31 C. Kutal and R. E. Sievers, *Inorg. Chem.*, 13 (1974) 897.
32 T. Fujinaga and S. Murai, *Anal. Chem. Aita*, 71 (1974) 141.
33 J. P. McKavenly and H. R. Freiser, *Anal. Chem.*, 29 (1957) 290.
34 J. F. Steinbach and J. H. Burns, *J. Amer. Chem. Soc.*, 80 (1958) 1839.
35 C. Veillon, in J. D. Winefordner (Editor), *Trace Analysis: Spectroscopic Methods for Elements*, Wiley, New York, 1976, p. 164.
36 A. K. De, S. M. Khopkar and R. A. Chalmers, *Solvent Extraction of Metals*, Van Nostrand Reinhold, New York, 1970, p. 46.